Physiological Ecology

Influence of Temperature, Nourishment, and Storage Period on the Longevity and Fecundity of the Mealybug Parasitoid, *Anagyrus* sp. nov. nr. *sinope* Noyes and Menezes (Hymenoptera: Encyrtidae)

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Environ. Entomol. 35(5): 1198-1207 (2006)

ABSTRACT Anagyrus sp. nov. nr. sinope Noyes and Menezes (Hymenoptera: Encyrtidae) is a candidate for biological control of the Madeira mealybug, *Phenacoccus madeirensis* Green (Hemiptera: Pseudococcidae). P. madeirensis is a serious pest of horticultural crops in the southeastern United States. The first experiment studied the effect of temperature (15, 20, 25, 30, and 35°C) and feeding treatment (starved, hydrated with distilled water, and nourished and hydrated with diluted honey) on adult longevity of Anagyrus sp. nov. nr. sinope. Nourished and hydrated female parasitoids survived an average of 52.8 d at 15°C, which was 14 and 8 times longer than starved and hydrated females at the same temperature, respectively, and 8 times longer than nourished and hydrated females at 35°C. The second experiment studied the influence of feeding treatment on the reproduction of Anagyrus sp. nov. nr. sinope at 25°C. Nourished and hydrated female parasitoids lived (mean = 14.2 d) and reproduced (mean = 7 d) for a longer duration, parasitized more hosts (mean = 16), and produced more offspring (mean = 45) than starved or hydrated females. The third experiment assessed the longevity and reproduction of Anagyrus sp. nov. nr. sinope after storage for various durations (0, 1, 4, 7, 14, 21 d) at 15 and 25°C. Parasitoids stored at 15°C had increased longevity and a decreased reproductive period but similar parasitism rates and fecundity to those stored at 25°C. The results suggested that the parasitoids could be stored at 15 and 25°C for up to 14 d without significant reduction in fecundity after release.

KEY WORDS biological control, food supplements, storage temperature, storage duration, *Phenacoccus madeirensis*

The Madeira mealybug, Phenacoccus madeirensis Green, is considered to be one of the most damaging pests in the production of ornamentals in greenhouses of the southeastern United States (Chong et al. 2003). P. madeirensis, together with other scale insect and mealybug species, caused an estimated \$71 million in losses to Georgia's ornamental production and maintenance in 2001 (Oetting et al. 2002). The species was described from specimens collected on the Madeira Island (Green 1923). However, Williams and Granara de Willink (1992) suggested that the species may have originated in tropical America and spread around the world through international trades. P. madeirensis feeds on members from >40 plant families, including many important field and horticultural crops (Ben-Dov 1994). Conventional chemical and cultural management tactics are insufficient in managing the mealybug populations and preventing extensive direct and indirect damage caused by the infestations.

Anagyrus sp. nov. nr. sinope Noyes and Menezes is currently being evaluated as a potential biological control agent of *P. madeirensis*. The parasitoid species originated as a local contamination from a colony of Anagyrus loecki Noyes and Menezes, which is another candidate biological control agent of P. madeirensis. Aspects of the biology, behavior, and parasitoid-host interactions of Anagyrus sp. nov. nr. sinope were studied by Chong (2005) (reported as A. loecki). Anagyrus sp. nov. nr. sinope is specific to P. madeirensis (Chong and Oetting 2006). The parasitoid also successfully develops and reproduces between 15 and 30°C, which is the temperature range often maintained in a greenhouse. The parasitoid also prefers to parasitize thirdinstar and prereproductive adult female P. madeirensis. Chong (2005) concluded that Anagyrus sp. nov. nr. sinope is potentially an effective augmentative biological control agent against *P. madeirensis* in greenhouse ornamental productions because of its host specificity, higher developmental and population growth rates than its target host under similar environmental conditions, and ability to locate the target host effectively.

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An understanding on the effects of temperature and food availability on the survival and reproduction of Anagyrus sp. nov. nr. sinope is essential for the development of rearing and shipping procedures that would ensure high survival rate and effectiveness of the released parasitoids. Many studies have shown that rising temperature significantly reduces longevity and fecundity of parasitoids (Sagarra et al. 2000, Matadha et al. 2004). Adult feeding is essential to the survival and reproduction of parasitoids (Jervis and Copland 1996, Jervis et al. 1996). Many adult hymenopteran parasitoids obtained carbohydrates from honeydew and floral and extra-floral nectar and proteins from host hemolymph and pollens. The nutrients consumed are used by adult parasitoids in somatic maintenance, reproduction, and as fuel for foraging or courtship activities. It is a common practice to add a food supplement and keep the parasitoids in cold storage to maintain high survival of parasitoids during shipment.

The objectives of this study were to answer the following questions. What is the interactive effect of temperature and nourishment on the longevity and fecundity of adult *Anagyrus* sp. nov. nr. *sinope*? Will the extended storage period compromise the efficiency and fecundity of *Anagyrus* sp. nov. nr. *sinope*?

Materials and Methods

Sources of Insects. *Phenacoccus madeirensis* were collected from an insectary colony maintained on sprouted russet potatoes (*Solanum tuberosum* L.) at the University of Georgia, Griffin Campus, Griffin, GA. Adult female mealybugs of body length 2–2.5 mm were selected for the experiments to standardize the host quality.

Anagyrus sp. nov. nr. sinope used in this study were collected from a laboratory colony maintained on P. madeirensis reared on sprouted potatoes at the Griffin Campus. The colony was established in May 2002 with parasitoids isolated from a greenhouse colony of A. loecki, which was maintained as candidate biological control agent of P. madeirensis. The two Anagyrus spp. are different in the number of progeny produced per mummy or brood size: A. loecki is strictly solitary, whereas *Anagyrus* sp. nov. nr. *sinope* is gregarious. Other morphological characteristics such as the coloration of flagellum and mesopleuron also distinguish Anagyrus sp. nov. nr. sinope as a distinct species (J. S. Noyes, personal communication). Anagyrus sp. nov. nr. sinope is believed to be a local contamination because the species was not detected in the original colony of A. loecki maintained at the University of Florida, Mid-Florida Research and Education Center, Apopka, FL (L. S. Osborne, personal communication). Mummies were collected, isolated individually in gelatin capsules (no. 1; Eli Lilly and Co., Indianapolis, IN), and incubated to adult emergence in an environmental chamber maintained at 25°C and 14:10

Specimens of *Anagyrus* sp. nov. nr. *sinope* are deposited at the Georgia Museum of Natural History,

University of Georgia, Athens, GA, and the Natural History Museum, London, UK.

Influence of Temperature and Feeding Treatment on Adult Longevity. Male and female adults of Anagyrus sp. nov. nr. sinope were subjected to five constant temperatures (15, 20, 25, 30, and 35°C) and three feeding treatments (starved, hydrated with only distilled water, and hydrated and nourished with 50% honey solution) arranged in a two-way factorial design. A 50% honey solution was prepared by diluting 100 ml of honey, purchased from a local grocery store, with the same volume of distilled water. Host feeding by Anagyrus sp. nov. nr. sinope had never been observed in either laboratory, greenhouse, or field conditions. As a result, we did not include hosts as separate nourishment.

Adult parasitoids were collected on emergence and individually released into a 1-dram glass vial, which was stoppered with a cotton ball to prevent the escape of parasitoids and allow ventilation. Water or honey solution was supplied on pieces of filter paper on the bottom of each glass vial. Parasitoids assigned to the starvation treatment received no food supplement. The parasitoids were kept at one of the five prescribed temperatures until death. The glass vials and filter papers were changed every week to prevent growth of mold. Immediately after their death, the left hind tibial lengths of all parasitoids were measured using an ocular micrometer fitted to a dissecting microscope. Body length is often correlated to adult longevity and fecundity of adult parasitoids (Jervis and Copland 1996). To assess whether body length and hind tibial length are correlated, five individuals were randomly selected from each temperature/feeding treatment combination, and their body length and hind tibial length were measured after death. In this experiment, 30 females and 15 males were prepared for each temperature/feeding treatment combination. Individuals that drowned in excess solution were excluded from statistical analyses.

The longevity of *Anagyrus* sp. nov. nr. *sinope* is the time elapsed between emergence and death of the adult parasitoids. The effects of temperature and feeding treatment on adult longevity of Anagyrus sp. nov. nr. sinope were analyzed with two-way analysis of variance (ANOVA), and the means were separated by Tukey's honestly significant difference (HSD) test at a significant threshold of 0.05 (PROC GLM; SAS Institute 1999). To assess the potential of hind tibial length as a meaningful predictor of body length, the body lengths of five individuals from each temperature/feeding treatment combination were first regressed against their tibial lengths (PROC REG; SAS Institute 1999). Individual adult longevity was regressed against hind tibial length of all parasitoids tested to test for the hypothesis of increased longevity with increased body size (PROC REG).

Influence of Feeding Treatment on Adult Reproductive Period, Lifetime Fecundity, and Progeny Sex Ratio. Female *Anagyrus* sp. nov. nr. *sinope* were collected and narcotized with CO₂ on emergence, after which their hind tibial lengths were measured with an

ocular micrometer. Thirty-six female parasitoids with hind tibial length of 0.40 mm were each paired with one adult male on emergence and randomly assigned to one of three feeding treatments (starved, hydrated, and hydrated and nourished). Each female Anagyrus sp. nov. nr. sinope was released into a petri dish (60 by 15 mm) supplied with a piece of filter paper wetted by the designated food solution and 10 adult female P. madeirensis. Female parasitoids subjected to the starvation treatment received only dry filter paper. The parasitoids were allowed to forage in the petri dishes for 24 h at 25°C, $75 \pm 5\%$ RH, and photoperiod of 10:14 (L:D) h. The relative humidity was maintained by sealing all petri dishes in a glass jar (25 by 20 cm) containing 200 ml of saturated sodium chloride solution. The parasitoids were removed and released into a new petri dish containing the same number of fresh mealybugs and food solution. This process continued until the death of the parasitoids.

After the exposure to parasitoids, the mealybugs in each petri dish were moved onto an excised chrysanthemum ($Dendrathema \times grandiflora$ Kitam.) leaf and incubated at 25°C until mummification. An excised chrysanthemum leaf was maintained in a petri dish (100 by 20 mm) by inserting its petiole through a hole at the bottom of the petri dish into a cup of water. The mummies were collected after 10 d, individually isolated in gelatin capsules, and incubated at 25°C until adult emergence. The emerged parasitoids were counted and sexed. Each feeding treatment was replicated 10 times.

Longevity was the duration between parasitoid emergence to death. The reproductive period was the duration between the first and the last day when eggs were deposited (confirmed by the formation of mummies). Proportion of males was used as a measurement of progeny sex ratio and was arcsine-transformed before statistical analyses. ANOVA was used to assess the effects of feeding treatment on the lifetime fecundity, longevity, reproductive period, and progeny sex ratio of *Anagyrus* sp. nov. nr. *sinope* (PROC GLM). When a significant difference among the means was detected, the means were separated by Tukey's HSD test.

Influence of Storage Temperature and Duration on Adult Reproductive Period, Lifetime Fecundity, and Progeny Sex Ratio. Two constant temperatures (15 and 25°C) and six storing durations (0, 1, 4, 7, 14, and 21 d) were arranged in a two-way factorial design with 10 replications for each temperature/duration combination. Adult female Anagyrus sp. nov. nr. sinope were collected on emergence, and only those with hind tibial length ≥0.4 mm were selected for the experiment. Each selected female parasitoid was paired with a single male parasitoid and released into a glass vial supplied with 50% honey solution and held at one of the two constant temperatures for the specific storing duration. Parasitoids that were assigned to 0-d storing duration treatment were fed with honey solution and used in the experiment immediately after collection.

At the end of the storage period, each female Anagyrus sp. nov. nr. sinope was released into a petri dish with 10 adult female P. madeirensis on an excised chrysanthemum leaf. The mealybugs were transferred onto the chrysanthemum leaf 16 h before the experiment to allow settlement and feeding. Streaks of honey were provided as food for the parasitoids. The parasitoids were allowed to forage for 24 h at 25°C and $92 \pm 8\%$ RH and a photo-cycle of 14:10 h (L:D). No specific efforts were given to maintain the relative humidity in this experiment. After 24 h, the parasitoids were removed and released into new petri dishes each containing 10 fresh hosts. This process was continued until the death of all parasitoids. The mealybugs were incubated at 25°C until mummification. Mortality of mealybugs caused by host feeding was considered negligible because the parasitoid was never observed to host feed in laboratory or greenhouse. The mummies were collected, isolated in gelatin capsules, and incubated at 25°C until adult parasitoid emergence. The parasitoids were counted and sexed on emergence. Parameters tested in the statistical analyses were calculated as described in the previous section. Adult longevity included the storage duration. Progeny sex ratio was arcsine-transformed before statistical analyses. The effects of storing temperature and duration on the lifetime fecundity, longevity, reproductive period, and progeny sex ratio of *Anagyrus* sp. nov. nr. *sinope* were analyzed with two-way ANOVA, followed by mean separation procedure with Tukey's HSD test (PROC GLM).

Results

Influence of Temperature and Feeding Treatment on Adult Longevity. Temperatures, feeding treatments, and their interactions significantly influenced adult longevity of both male and female Anagyrus sp. nov. nr. sinope (Table 1). Within each temperature treatment, hydrated and nourished males and females survived considerably longer than starved or hydrated individuals. No difference was observed between the longevity of starved and hydrated parasitoids. Parasitoids fed with the same food survived longer at lower holding temperatures than at higher temperatures. The highest average longevity for females was 52.8 d when fed with 50% honey solution at 15°C, and the lowest was ≈ 1 d when starved or provided with only distilled water at 35°C. Similarly, hydrated and nourished males survived the longest at 15°C (≈32 d), whereas starved and hydrated males survived the shortest at 35°C (1.2-1.3 d). Males had similar or shorter longevity than females in all temperature/ feeding treatment combination. Extremely high temperature (35°C) seemed to be detrimental to the survival of adult *Anagyrus* sp. nov. nr. *sinope*. None of the females and males subjected to 35°C treatment survived >7 and 4 d, respectively, regardless of food

Female body lengths ranged from 0.8 to 1.45 mm and were larger than males (0.7–1.1 mm). Hind tibial length was a sufficient predictor of female body

Table 1. Means ± SEM of longevity (in days) of female and male Anagyrus sp. nov. nr. sinope adults subjected to various feeding treatments at five constant temperatures

| T. (90) | P. 1 | Longevity | | | |
|---------------------------------|--|-----------------------------|--------------------------|--|--|
| Temperature (°C) | Feeding treatment | Female | Male | | |
| 15 | Starved Hydrated | $3.9 \pm 0.2 \text{bA}$ | $4.4 \pm 0.3 \text{bA}$ | | |
| | Hydrated | $6.8 \pm 0.3 \mathrm{bA}$ | $5.3 \pm 0.6 \text{bA}$ | | |
| | Hydrated and nourished | $52.8 \pm 4.6 aA$ | $32.2 \pm 3.6 aA$ | | |
| 20 | Starved | $2.5 \pm 0.1 \mathrm{bB}$ | $2.2 \pm 0.2 \text{bB}$ | | |
| | Hydrated | $3.3 \pm 0.2 \text{bAB}$ | $3.5 \pm 0.2 \text{bB}$ | | |
| | Hydrated and nourished | $32.5 \pm 1.2aB$ | $20.5 \pm 1.2 aB$ | | |
| 25 | Starved | $1.9 \pm 0.1 \mathrm{bC}$ | $1.8 \pm 0.2 \text{bBC}$ | | |
| | Hydrated | $2.5 \pm 0.1 \mathrm{bBC}$ | $2.3 \pm 0.2 bC$ | | |
| | Hydrated and nourished | $23.4 \pm 1.7 \mathrm{aBC}$ | $15.7 \pm 2.8 aB$ | | |
| 30 | Starved | $1.6 \pm 0.1 \mathrm{bC}$ | $1.7 \pm 0.1 \text{bBC}$ | | |
| | Hydrated | $1.8 \pm 0.1 \text{bCD}$ | $2.1 \pm 0.1 \text{bC}$ | | |
| | Hydrated and nourished | $15.8 \pm 1.1 \mathrm{aCD}$ | $9.1 \pm 1.0 aC$ | | |
| 35 | Starved | $1.2 \pm 0.1 \mathrm{bC}$ | $1.2 \pm 0.1 bC$ | | |
| | Hydrated | $1.2 \pm 0.1 \mathrm{bD}$ | $1.3 \pm 0.1 bC$ | | |
| | Hydrated and nourished | $6.5 \pm 0.8 \mathrm{aD}$ | $3.9 \pm 0.3 aD$ | | |
| ANOVA F values | • | | | | |
| Temperature | | 68.92^{a} | 119.57^{a} | | |
| Feeding treatment | | 549.44^{a} | 301.66^a | | |
| Temperature × feeding treatment | | 38.28^{a} | 39.38^{a} | | |

The parasitoids were starved (no food, no water), hydrated with distilled water, or hydrated and nourished with honey solution. Longevity is the duration between adult emergence and death.

^a $P \le 0.0001$ (ANOVA, $\alpha = 0.05$).

length, accounting for 70% of the variation (Fig. 1A). Hind tibial length of males was not as good a predictor of body length. Although the male hind tibial length showed a significantly positive relationship to body length, it only explained 36% of variation in the observed data (Fig. 1B). Because there were significant interactions of temperature and feeding treatment on adult longevity, the possible influence of body size (using hind tibial length as a surrogate) on longevity was examined separately for each temperature and feeding treatment combinations. Significant regressions ($P \le 0.05$) between hind tibial length and longevity were detected in several temperature/feeding treatment combinations, especially for starved or hydrated females ≤30°C (Table 2). Hydrated and nourished males and females generally showed insignificant relationships between hind tibial length and adult longevity. Hind tibial length did not account for >50% of the variation in most temperature/feeding treatment combinations, except for hydrated males at 15°C and hydrated and nourished males at 25°C (Table 2).

Influence of Feeding Treatments on Lifetime Fecundity, Reproductive Period, and Progeny Sex Ratio. The starved female Anagyrus sp. nov. nr. sinope lived and reproduced for ≈ 3 d, which was 1–2 d shorter than those fed on distilled water (Fig. 2A; $F_{2,28} = 58.59$, P < 0.0001). The longevity and reproductive periods of hydrated and nourished parasitoids were more than three and two times longer, respectively, than that of starved parasitoids (Fig. 2; for reproductive period, $F_{2,28} = 24.60$, P < 0.0001). Because of the reduction in life expectancy, many starved females failed to parasitize any mealybug. Only ≈ 4 mealybugs were parasitized by the starved or hydrated parasitoids over the

reproductive period compared with 16 by the hydrated and nourished females (Fig. 3; $F_{2,28} = 43.63$, P < 0.0001).

Differences in the parasitism efficiencies among parasitoids subjected to the three feeding treatments resulted in significant differences in the number of progeny produced (Fig. 4). Hydrated and nourished female parasitoids produced 30 females and 15 males. which was 4 and 3.6 times higher than those produced by starved females, respectively (for female progeny, $F_{2.28} = 37.45$, P = 0.0001; for male progeny, $F_{2.28} =$ 23.26, P < 0.0001). The total number of progeny produced by parasitoids fed with honey solution was 45 offspring, which was about four times higher than that by the starved parasitoids. Secondary sex ratios were not significantly different among the feeding treatments $(F_{2.28} = 0.46, P = 0.6373)$. The proportion of male progeny was 0.38 by starved parasitoids, 0.35 by hydrated females, and 0.33 by hydrated and nourished parasitoids.

Influence of Storage Temperature and Duration on Lifetime Fecundity, Reproductive Period, and Progeny Sex Ratio. Storage temperature and duration affected the reproductive period and longevity of Anagyrus sp. nov. nr. sinope (Table 3). No significant interactions between storing temperature and duration were detected. At 15°C, the reproductive period was reduced from ≈ 11 to 5 d for parasitoids held for 0–21 d, respectively, whereas the longevity increased from ≈ 15 to 28 d within the same holding durations. Similar results were reported for both reproductive period and longevity of Anagyrus sp. nov. nr. sinope at 25°C. Significant differences in reproductive period and longevity between the two storage temperatures

Mean longevity of females or males followed by the same small letter are not significantly different among feeding treatments within a specific temperature. Mean longevity of females or males followed by the same capital letter are not significantly different among temperature within a specific feeding treatment (Tukey's HSD, $\alpha = 0.05$).

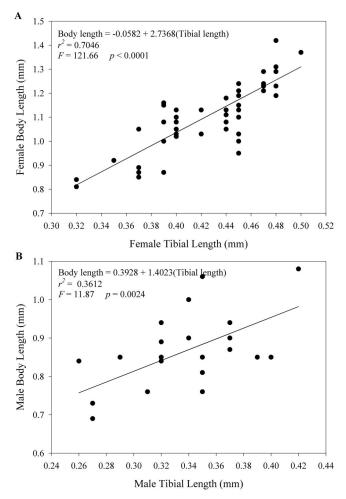


Fig. 1. Body length (mm) of female (A) and male (B) Anagyrus sp. nov. nr. sinope as a function of hind tibial length (mm). The solid lines are the results of regression analyses.

were detected only in parasitoids stored for 21 d where individuals stored at 15°C reproduced and survived longer than those at 25°C. No significant differences between the two storage temperatures were detected in the number of hosts parasitized, the fecundity, and the progeny sex ratio (Table 3).

Anagyrus sp. nov. nr. sinope stored for >14 d parasitized significantly fewer P. madeirensis over a life-

time and subsequently produced fewer offspring than those stored for shorter durations (0–7 d; Table 3). Parasitoids that were allowed to forage immediately after emergence (0 d) parasitized 24–25 *P. madeirensis* at 15 and 25°C, which were three and eight times as many as those held for 21 d, respectively. The total number of progeny produced by parasitoids held for 0 d at 15°C was 62.8, which was 1.5 and 3 times more

Table 2. Regression coefficients (r^2) and P values for the linear regression analyses between tibial length and longevity of female and male Anagyrus sp. nov. nr. sinope adults subjected to various temperature/feeding treatment combinations

| | Feeding treatment | Temperatures (°C) | | | | | | | | | |
|--------|------------------------|-------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| Sex | | 15 | | 20 | | 25 | | 30 | | 35 | |
| | | r^2 | P | r^2 | P | r^2 | P | r^2 | P | r^2 | P |
| Female | Starved | 0.0970 | 0.0881 | 0.3297 | 0.0002 | 0.1034 | 0.0831 | 0.1857 | 0.0174 | 0.1295 | 0.2062 |
| | Hydrated | 0.1850 | 0.0157 | 0.2015 | 0.0214 | 0.2353 | 0.0120 | 0.2644 | 0.0051 | 0.0379 | 0.5660 |
| | Hydrated and nourished | 0.0449 | 0.2698 | 0.0695 | 0.0880 | 0.0530 | 0.2793 | 0.2057 | 0.0200 | 0.0865 | 0.3294 |
| Male | Starved | 0.1557 | 0.2023 | 0.1562 | 0.1297 | 0.0001 | 0.9725 | 0.1894 | 0.1049 | 0.3792 | 0.0001 |
| | Hydrated | 0.5485 | 0.0143 | 0.1942 | 0.0999 | 0.0010 | 0.9354 | 0.2522 | 0.0803 | 0.0532 | 0.1968 |
| | Hydrated and nourished | 0.1758 | 0.1993 | 0.0586 | 0.1612 | 0.9442 | 0.0057 | 0.0679 | 0.3681 | 0.0438 | 0.3158 |

The parasitoids were starved (no food, no water), hydrated with distilled water, or hydrated and nourished with honey solution.

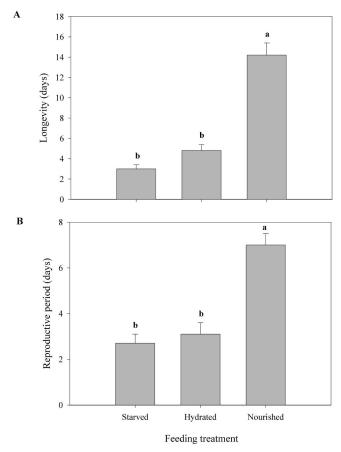


Fig. 2. Longevity (A) and reproductive period (B) of Anagyrus sp. nov. nr. sinope subjected to various feeding treatments at 25° C and $75 \pm 5\%$ RH. The parasitoids were starved (no food, no water), hydrated with distilled water, or hydrated and nourished with honey solution. Longevity includes prereproductive and reproductive periods. Bars topped with the same letter are not significantly different.

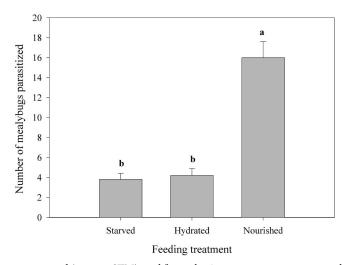


Fig. 3. Number of hosts parasitized (mean \pm SEM) in a lifetime by Anagyrus sp. nov. nr. sinope subjected to various feeding treatments at 25°C and 75 \pm 5% RH. The parasitoids were starved, hydrated with distilled water, or hydrated and nourished with honey solution. Bars topped with the same letter are not significantly different.

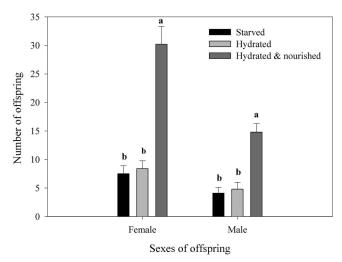


Fig. 4. Number of female and male offspring (mean \pm SEM) produced in a lifetime by Anagyrus sp. nov. nr. sinope subjected to various feeding treatments at 25°C and 75 \pm 5% RH. The parasitoids were starved, hydrated with distilled water, or hydrated and nourished with honey solution. Bars topped with the same letter are not significantly different.

than the parasitoids stored for 14 and 21 d, respectively, at the same temperature. Parasitoids stored for 0–7 d produced similar number of offspring at 15 and 25°C. Progeny secondary sex ratios (reported as proportion of males) ranged from 0.32 to 0.37 and were not significantly different among the different storage durations at both temperatures (Table 3).

Discussion

Most adult parasitoids require food, such as host hemolymph, host honeydew, nectar, pollen, or other carbohydrate- or protein-rich substitutes, to maintain somatic and reproduction fitness and to obtain energy for foraging and reproductive activities. Adult longevity often declines in the absence of food sources (Jervis et al. 1992). When provided with a carbohydrate source in the form of honey solution, both sexes of *Anagyrus* sp. nov. nr. *sinope* lived 3–13 times longer than those provided with only distilled water or starved. Provisions of honey solution increased the longevity of the encyrtid parasitoid *Tachinaephagus zealandicus* Ashmead by one to three times of those individuals fed with only water (Ferreira de Almeida et al. 2002a). Longevity of *Coccidoxenoides perminutus* Girault (Hymenoptera: Encyrtidae) was highest when

Table 3. Longevity, reproductive time, no. of hosts parasitized over lifetime, no. of offspring, and sex ratio (means \pm SEM) of female Anagyrus sp. nov. nr. sinope subjected to various storage temperature and duration combinations in a relative humidity of 90 \pm 5%

| Temperature (°C) | Duration (days) | Longevity (days) | Reproductive period (days) | Number hosts | Nu | Sex ratio | | | | |
|------------------------|-----------------|----------------------------|----------------------------|------------------|-------------------|------------------|-------------------|-----------------|--|--|
| | | | | parasitized | Female | Female Male | | (proportion ♂) | | |
| 15 | 0 | 15.1 ± 1.3 cA | 11.1 ± 1.1aA | $23.9 \pm 1.7a$ | $41.5 \pm 2.7a$ | $21.3 \pm 1.5a$ | $62.8 \pm 3.6a$ | 0.34 ± 0.01 | | |
| | 1 | 15.5 ± 1.4 cA | $11.9 \pm 1.3aA$ | $24.1 \pm 2.8a$ | $41.3 \pm 3.0a$ | $20.1 \pm 1.6a$ | $61.4 \pm 4.6a$ | 0.33 ± 0.01 | | |
| | 4 | $17.7 \pm 1.2 bcA$ | $11.8 \pm 1.3 aA$ | $23.1 \pm 2.2a$ | $41.0 \pm 3.8a$ | $22.1 \pm 2.0a$ | $63.1 \pm 5.8a$ | 0.35 ± 0.01 | | |
| | 7 | $22.0 \pm 1.9 aA$ | $10.8 \pm 1.9 abA$ | $19.3 \pm 2.8a$ | $34.1 \pm 4.1ab$ | $18.5 \pm 2.7a$ | $52.6 \pm 6.8ab$ | 0.35 ± 0.01 | | |
| | 14 | $24.5 \pm 2.0 aA$ | $9.2 \pm 2.0 abA$ | $14.6 \pm 2.5ab$ | $25.6 \pm 3.3 bc$ | $14.2 \pm 2.2ab$ | $39.8 \pm 5.4 bc$ | 0.35 ± 0.01 | | |
| | 21 | $27.9 \pm 1.2 aA$ | $4.9 \pm 0.9 \text{bA}$ | $7.9 \pm 1.6b$ | $12.7 \pm 1.9c$ | $7.4 \pm 1.2b$ | $20.1 \pm 3.1c$ | 0.36 ± 0.02 | | |
| 25 | 0 | $14.2 \pm 1.0 cA$ | $10.4 \pm 0.9 abA$ | $24.9 \pm 1.7a$ | $42.9 \pm 2.9a$ | $20.5 \pm 1.5a$ | $63.4 \pm 4.2a$ | 0.32 ± 0.01 | | |
| | 1 | $15.1 \pm 1.0 cA$ | $10.8 \pm 1.0 aA$ | $24.8 \pm 2.1a$ | $44.2 \pm 3.9a$ | $20.9 \pm 1.6a$ | $65.1 \pm 4.7a$ | 0.32 ± 0.02 | | |
| | 4 | $16.5 \pm 1.7 bcA$ | $10.4 \pm 1.6 abA$ | $23.3 \pm 2.7a$ | $44.2 \pm 4.1a$ | $21.5 \pm 1.6a$ | $65.7 \pm 5.1a$ | 0.33 ± 0.02 | | |
| | 7 | $18.0 \pm 0.9 bcA$ | $8.1 \pm 0.5 abA$ | $17.8 \pm 1.4ab$ | $35.6 \pm 3.2ab$ | $18.3 \pm 1.8ab$ | $53.9 \pm 4.7ab$ | 0.34 ± 0.02 | | |
| | 14 | $20.5 \pm 1.4 abA$ | $6.5 \pm 1.0 \mathrm{bA}$ | $12.4 \pm 1.4b$ | $25.0 \pm 3.5b$ | $12.8 \pm 1.8b$ | $37.8 \pm 5.2b$ | 0.34 ± 0.01 | | |
| | 21 | $23.3 \pm 0.5 \mathrm{aB}$ | $1.7 \pm 0.3 \mathrm{eB}$ | $3.3 \pm 0.7e$ | $5.9\pm1.0c$ | $3.3 \pm 0.6c$ | $9.2\pm1.5c$ | 0.37 ± 0.04 | | |
| | | ANOVA F values | | | | | | | | |
| Temperature | | 10.11^{a} | 7.35^{a} | 0.79^{c} | 0.02^{c} | 1.09^{c} | 0.08^{c} | 0.33^{c} | | |
| Duration | | 19.75^{b} | 11.91^{b} | 26.09^{b} | 34.30^{b} | 26.16^{b} | 34.49^{b} | 0.83^{c} | | |
| Temperature × duration | | 0.93^{c} | 0.33^{c} | 0.53^{c} | 0.66^{c} | 0.43^{c} | 0.63^{c} | 0.19^{c} | | |

Longevity included storage duration and reproductive time after storage.

Means followed by the same small letter within each temperature treatment are not significantly different among the holding durations. Means followed by the same capital letter within each storage period are not significantly different between the temperature treatments (Tukey's HSD, $\alpha = 0.05$).

 $^{{}^{}a}P \le 0.01; {}^{b}P \le 0.0001; {}^{c}P > 0.05 \text{ (ANOVA, } \alpha = 0.05).$

the parasitoids were provided with honey solution compared with parasitoids provided no food, flowers, nectar, or mealybug honeydew (Davies et al. 2004). Distilled water apparently did not provide any nutritional value to Anagyrus sp. nov. nr. sinope because the longevity of the hydrated parasitoids was identical to that of the starved parasitoids. However, González-Hernández et al. (2005) showed that the provision of moisture and carbohydrate has a synergistic effect to the survival of adult parasitoids. The longevity of female Anagyrus ananatis Gahan (Hymenoptera: Encyrtidae) was extended to ≈ 28 d when fed with 50% honey solution, and those provided with only pure honey lived for 19 d (González-Hernández et al. 2005).

Many examples showed that longevity of adult parasitoids generally decreases with increase in temperature. Adult longevity of Anagyrus sp. nov. nr. sinope decreased significantly with incremental increase in temperature. The longevity of Anagyrus kamali Moursi fed with honey decreased from 40.3 d at 20°C to 29 d at 27°C (Sagarra et al. 2000). Similar results were also reported across order Hymenoptera from Anagyrus pseudococci (Girault), Leptomastix dactylopii Howard, Leptomastidea abnormis (Girault) (all Encyrtidae) (Tingle and Copland 1989), Anagrus atomus L. (Mymaridae) (Agboka et al. 2004), Lysiphlebia citricida Shuja-Uddin (Aphidiidae) (Liu and Tsai 2002), Encarsia citrina Craw (Aphelinidae) (Matadha et al. 2004), Catolaccus hunteri Crawford (Pteromalidae) (Seal et al. 2002), and Telenomus cyamophylax Polaszek (Scelionidae) (Foerster and Butnariu 2004). The longevity of red imported fire ant (Solenopsis invicta Buren [Hymenoptera: Formicidae]) parasitoid *Pseudacteon tricuspis* Borgmeier (Diptera: Phoridae) was reduced at a higher temperature (Chen et al. 2005).

There was also a significant interactive effect of temperature and feeding treatment on the longevity of adult *Anagyrus* sp. nov. nr. *sinope*. Adult *Anagyrus* sp. nov. nr. sinope fed the same food survived longer at lower temperatures than higher temperature. Sagarra et al. (2000) and Liu and Tsai (2002) reported similar phenomena. However, the authors did not perform analyses on the interactive effects of temperature and food supplements. A. kamali fed with honey at 20°C lived significantly longer than the starved females at the same temperature (40 and 2 d, respectively) (Sagarra et al. 2000). Honey-fed A. kamali also survived longer at 20°C than at 27°C (40 and 29 d, respectively). Similarly, the aphid parasitoid *L. mirzai* that was held at lower temperatures (5 and 10°C) and supplied with honey solution survived longer than those held at higher temperatures (15–30°C) and fed with only water or provided with no food (Liu and Tsai 2002).

Besides the availability of food sources, an additional factor that influences adult longevity is the body size of the parasitoids, measured through extrapolation from the length of body parts such as body, head, or tibia (Jervis and Copland 1996). Body size, measured by hind tibial length, had been shown to be an excellent indicator of the fitness of *A. kamali:* the

larger females lived and reproduced for a longer duration and produced more female offspring per day and over the lifetime than the smaller females (Sagarra et al. 2001). However, the body size (using hind tibial length as surrogate) of *Anagyrus* sp. nov. nr. sinope was not correlated with the adult longevity in many cases despite similar body size with A. kamali. Anagyrus sp. nov. nr. sinope of various body sizes survived equally well within each temperature/feeding treatment combination. Recently, similar findings were reported for Celatoria compressa (Diptera: Tachinidae), in which body size did not correlate with its longevity and fecundity (Zhang et al. 2004). Female Anagyrus sp. nov. nr. sinope may have higher energy reserves because of their larger body size and thus lived longer than the males. The potential benefit of larger body size for adult parasitoid longevity seemed to break down at 35°C, where all parasitoids lived for only a few days regardless of their body sizes.

Adult parasitoids often produce more progeny when fed with a more nutritious food source, such as host hemolymph and liquid sugar sources (Jervis et al. 1992). Female parasitoids hydrated and nourished with honey solution lived longer and were more active in searching, thus allowing them to parasitize more hosts and produce more progeny. Compared with female Anagyrus sp. nov. nr. sinope deprived of all food sources, hydrated and nourished females lived and reproduced about five times longer and produced seven times more progeny. Ferreira de Almeida et al. (2002b) reported that the hydrated and nourished T. zealandicus killed three times more muscoid fly hosts and produced four times more progeny per female than when no food was provided. A honey solution generally does not provide a significant amount of protein. Therefore, the increase in fecundity of both Anagyrus sp. nov. nr. sinope and T. zealandicus was most likely caused by an increase in energy, and the subsequent increase in foraging activities, from ingestion of carbohydrates. Similarly, the egg parasitoid Ooencyrtus nezarae Ishii (Hymenoptera: Encyrtidae) fed with honey spent almost five times as much time in searching for hosts than those provided with only water (Teraoka and Numata 2000).

Several recent studies have shown that fecundity peaked at some medium temperature (Seal et al. 2002, Agboka et al. 2004, Foerster and Butnariu 2004, Matadha et al. 2004). We did not observe such a pattern in *Anagyrus* sp. nov. nr. *sinope*. The decrease in adult longevity and reproductive period of *Anagyrus* sp. nov. nr. *sinope*, however, was consistent with results from other studies. Both total and reproductive longevities of *C. hunteri* (Seal et al. 2002), *A. atomus* (Agboka et al. 2004), *T. cyamophylax* (Foerster and Butnariu 2004), and *E. citrina* (Matadha et al. 2004) decreased significantly with an increase in temperature.

Longer storage periods increased the longevity but decreased the fecundity, reproductive period, and number of *P. madeirensis* parasitized by *Anagyrus* sp. nov. nr. *sinope*. Sagarra et al. (2000) obtained similar result where the increase in adult longevity of *A. kamali* was accompanied by a decrease in reproductive period. The survival of the immatures and adults of A. ananatis were also significantly reduced when stored at 10.1°C, which was below the developmental threshold (Pandey and Johnson 2005). Preconditioning of the parasitoids at 14.8°C seemed to improve the survival and fecundity of A. anantis. Storage did not negatively affect fecundity of Anagyrus sp. nov. nr. *sinope* until the storing duration exceeded 14 d. The lifetime fecundity of A. kamali was not different among the five storage periods (Sagarra et al. 2000). A contradictory result was obtained from another encyrtid parasitoid T. zealandicus where the females parasitized the highest number of hosts and produced the largest number of progeny after the parasitoids were held for 6 d at 15°C, and the fecundity remained relative high after 12 and 24 d (Ferreira de Almeida et al. 2002b). The difference between Anagyrus spp. and T. zealandicus may lay in the difference in their ovigeny (i.e., the classification of parasitoids based on the fraction of potential lifetime complement of eggs that is mature on adult emergence, as in Flanders 1950 and Jervis et al. 2001): Anagyrus spp. are synovigenic, whereas T. zealandicus are proovigenic. For proovigenic parasitoids, which emerge with full complement of their eggs, the females do not have to lay eggs before more eggs can be matured. Thus, after a few days of obtaining nutrients and building up an energy reserve, T. zealandicus were able to reproduce at full capacity for the rest of its life. For synovigenic parasitoids, however, the females have to lay the existing complement of eggs before the next batch can be matured. As a result, A. kamali and Anagyrus sp. nov. nr. sinope that were kept for longer durations were not able to mature and deposit more eggs before their lifespan ended.

The results of this study suggest that the effectiveness, longevity, and reproduction of *Anagyrus* sp. nov. nr. *sinope* could be greatly enhanced with provisions of carbohydrate sources, e.g., honey solution, within a moderate range of temperature (15–30°C). Rearing of *Anagyrus* sp. nov. nr. *sinope* will benefit from provisions of honey solution in the colony. Hydrated and nourished parasitoids not only survived longer than starved or hydrated only individuals, they were also more active in foraging and producing more progeny.

Adult Anagyrus sp. nov. nr. sinope provided with honey solution could be stored at 15°C for up to 14 d without significant depreciation of its fecundity and survival. Thus, the parasitoids can be held at low temperature for a long period of time in situations when the host population is low and allow augmentation of the parasitoid colony when the host population becomes larger again. The parasitoids can be collected, stored, and shipped within a week after emergence and remain highly fecund. The ability to store the parasitoids will allow the accumulation of a larger number of parasitoids for releases to ensure a better chance of establishment and success of the biological control program.

Acknowledgments

J. Noyes of the Natural History Museum, London, identified *Anagyrus* sp. nov. nr. *sinope*. K. Braman, J. Ruberson, R. Matthews, and M. van Iersel of the University of Georgia and two anonymous reviewers provided helpful comments to an earlier draft of the manuscript. L. Osborne of the University of Florida discussed the origin of *A. loecki*. M. Townsend, R. Harris, and S. Stevens provided technical assistance. Yoder Brothers (Barberton, OH) generously supplied chrysanthemum cuttings used in this study.

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Received for publication 25 January 2006; accepted 21 June 2006.